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Biosynthetic studies of versipelostatin, a novel 17-membered α-tetronic acid involved macrocyclic compound isolated from *Streptomyces versipellis*

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Abstract—Versipelostatin, a novel microbial metabolite consisting of a 17-membered macrocyclic aglycone with an α-acyltetronic acid functional group and sugar moieties was shown to be biosynthesized through a polyketide intermediate and the involvement of glyceric acid.

Versipelostatin (**1**), a metabolite of *Streptomyces versipellis* 4083-SVS6, isolated as a GRP78 molecular chaperone down-regulator (Fig. 1).[1] GRP78 acts as a molecular chaperone in endoplasmic reticulum (ER) by associating transiently with incipient proteins to facilitate protein folding.[2–4] Overexpression of GRP78 enables solid tumor cells to grow in hypoxic and glucose starved condition, which is the characteristic circumstance of the core of solid tumor.[5] Thus, specific inhibitors of GRP78 induction are expected to be promising antitumor agents.[6,7] Our screening program resulted in the isolation of **1** as the first inhibitor of the GRP78 expression. In addition to the attractive biological activities, **1** is also structurally interesting compound which is the first example consisting of the 17-membered macrocyclic skeleton with the α-acyltetronic acid moiety. Most of the tetronic acid containing compounds is the family of tetrocarcin,

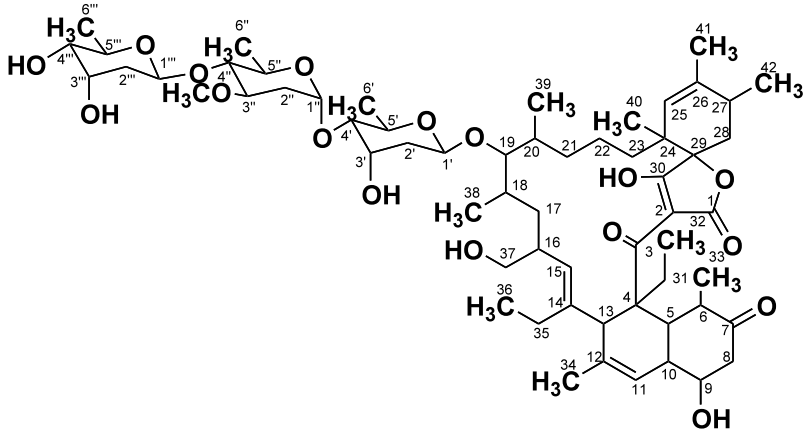

Figure 1. Structure of versipelostatin (**1**).

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which consist of 13-membered macrocyclic ring. Interestingly, 13-membered macrocyclic α-acyltetronic acid compounds showed robust cytotoxicity but could not mimic the activity of **1**, which specifically exhibited cytotoxic effects under hypoglycemic condition. Likewise, the number of macrocyclic skeleton seems to be critical to exert specific biological activities. Thus, the biosynthetic studies of **1** may serve important information to prepare derivatives of **1** by employing genetic engineering. Moreover, even the partial biosynthetic pathway of tetronic acid containing macrocyclic compounds were examined,[8] origin of the α-acyltetronic acid moiety in the secondary metabolites of actinomycetes has never been reported. Besides the biosynthetic studies on the α-acyltetronic acid moieties of plant metabolites were partially carried out, its detailed experiments were only performed in acaterin, a metabolite from *Pseudomonas* sp. A92.[9,10] In this regard, we have been very much interested in the biosynthetic mechanism of **1**. We report herein the biosynthetic studies of **1** by means of feeding experiments using ^{13}C-labeled precursors.

Although, the biosynthesis of the α-acyltetronic acid moieties in caloric acid and protoanemonin were reported to involve C_4 compounds in TCA cycle, succi-

Table 1. Incorporation ratio of [1-^{13}C]acetate, [3-^{13}C]propionate, [1-^{13}C]butyrate and [1,2,3-$^{13}C_3$]glycerol, and ^{13}C–^{13}C coupling constants observed with [1,2-$^{13}C_2$]acetate in the aglycone of versipelostatin

No.	δ_C	Relative enrichment*			J_{C-C} (Hz)	
		[1-^{13}C]acetate	[3-^{13}C]Propionate	[1-$^{13}C_1$]Butyrate	[1,2-$^{13}C_2$]Acetate	[1,2,3-C_3]Glycerol
1	166.4	1.9	0.6		78	78
2	103.2	1.0	0.7		78	78
3	205.1	1.9	0.7	6.0	45	45
4	58.6	1.0	0.9		45	45
5	39.3	1.3	0.9			
6	49.8	1.2	0.6			
7	209.6	2.3	0.7		37	37
8	50.1	1.1	0.6		37	37
9	71.3	2.6	0.7		36	36
10	47.2	1.1	0.6		36	36
11	120.8	1.1	0.8			
12	134.2	1.2	0.7			
13	60.0	2.8	0.6	10.6	39	39
14	139.3	1.2	0.9		39	39
15	135.9	1.2	0.8			
16	37.9	1.1	0.6			
17	32.2	1.1	0.8			
18	35.4	1.2	0.8			
19	91.1	1.1	0.7			
20	29.2	1.0	0.8			
21	32.3	2.6	0.8		34	34
22	20.0	1.0	0.7		34	34
23	34.6	1.1	0.8			
24	41.4	1.0	0.7			
25	126.6	1.1	0.8			
26	135.3	0.8	0.6			
27	31.1	3.0	0.8		35	35
28	36.9	1.1	0.7			35**
29	87.3	1.3	0.6			35, 44**
30	201.8	1.0	0.5			44**
31	23.4	2.4	0.7		34	34
32	12.4	1.2	1.0		34	34
33	16.3	1.2	3.2			
34	22.8	1.2	2.7			
35	21.8	2.4	0.8		34	34
36	14.6	1.2	1.1		34	34
37	64.7	1.2	2.6			
38	17.1	1.2	2.8			
39	22.3	1.3	3.2			
40	20.8	1.3	2.9			
41	21.4	1.3	2.6			
42	19.4	1.2	0.8		35	35

ppm from internal TMS in $CDCl_3$ (125 MHz).

* Normalized to C2.

** These signals showed typical AMX type coupling pattern.

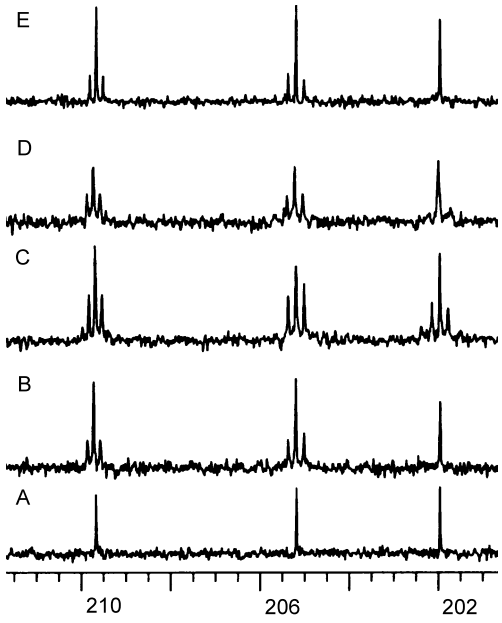
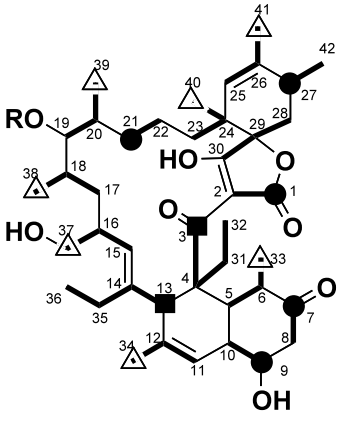


Figure 2. ^{13}C NMR spectra (125 MHz) of **1**. (A) Control. (B) Derived from [1,2-$^{13}C_2$]acetate. (C) Derived from [1,2,3-$^{13}C_3$]glycerol. (D) Decoupling experiment of **1** derived from [1,2,3-$^{13}C_3$]glycerol irradiating at C-29 position. (E) Derived from [1,2,3-$^{13}C_3$]pyruvate.

nate and α-ketoglutaric acid, respectively,[11,12] most of α-acyltetronic acid containing compounds appear to be biosynthesized via a C_3 unit. To confirm that the α-acyltetronic acid moiety in **1** originates from C_3 unit, *Streptomyces versipellis* 4083-SVS6 was cultivated as described previously and 24 h after inoculation, [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate were added as polyketide precursor at the level of 1.0 mg/ml as a final concentration. The labeled versipelostatins were isolated by solvent extraction followed by preparative TLC using $CHCl_3$–MeOH (20:1) as a developing solvent. The ^{13}C NMR spectrum of **1** labeled with [1-^{13}C]acetate revealed the enrichment of nine carbon signals in the aglycone moiety (C-1, C-3, C-7, C-9, C-13, C-21, C-27, C-31 and C-35) with the incorporation ratio being 1.9 to 3.0 as shown in Table 1. Furthermore, the direction of the incorporated acetate units were confirmed by the feeding experiment of [1,2-$^{13}C_2$]acetate as shown in Figure 2B and Figure 3. **1** partially consists of branched skeleton in its aglycone which suggests the involvement of the incorporation of propionate unit precursors. Thus, [3-^{13}C]propionate was further tested for feeding experiment. The ^{13}C NMR spectrum of the resulting [3-^{13}C]propionate labeled compound showed enrichment at C-33, C-34, C-37, C-38, C-39, C-40 and C-41 positions, indicating seven methyl carbon atoms were derived from the methyl residue of propionate. The incorporated pattern of [1-^{13}C]acetate suggested that the sequences between C-3, C-4, C-31 and C-32, and additionally that of C-13, C-14, C-35 and C-36 were biosynthesized from butyric acid predicted from its chemical structure. Strong enhancement of C-3 and C-13 in [1-^{13}C]butyrate labeled **1** proved that these substructures were derived from butyrate as C_4 units. Based on the results presented vide supra, it was concluded that the aglycone of **1** was synthesized via the polyketide pathway from five acetate, seven propionate and two butyrate units. Thus, the remaining unit in the α-acyltetronic acid moiety was deduced to originate from three-carbon unit. As mentioned above, the α-acyltetronic acid moiety in acaterin was derived from an acetate and glycerol units,[9] we next employed [1,2,3-$^{13}C_3$]glycerol for feeding experiment (Fig. 2C). Since glycerol was metabolized to form acetate, it was indirectly incorporated into **1** as acetate units. J_{C-C} couplings recognized in C-7 (δ_C 209.6, J_{C-C} = 37 Hz) and C-3 (δ_C 205.1, J_{C-C} = 45 Hz) showed indirectly incorporation originated from [1,2-$^{13}C_2$]acetate (Fig. 2C) of which the same coupling constants were also observed in Figure 2B. To the contrary, the analysis of J_{C-C} coupling constants proved that [1,2,3-$^{13}C_3$]glycerol was directly incorporated as a C_3 unit into remaining unassigned carbons C-28, C-29 and C-30 which consists of the α-acyltetronic acid moiety (Fig. 2C, $J_{C28-C29}$ = 35 Hz, $J_{C29-C30}$ = 44 Hz). These couplings were further confirmed by the decoupling experiment irradiating at C-29 (Fig. 2D). Although pyruvic acid, which is thought to be the downstream metabolite of glycerol, is the second



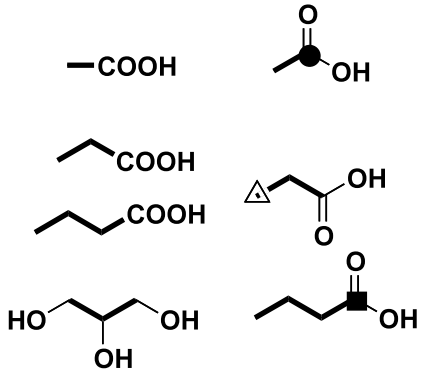


Figure 3. Labeling pattern of **1**.

candidate for the precursor of C_3 unit, it was not incorporated into tetronic acid substructure in acaterin.[9] As observed in acaterin, [1,2,3-$^{13}C_3$]pyruvic acid was neither incorporated into the C_3 unit of α-acyltetronic acid moiety in **1**. While acetate precursors, which were metabolized from [1,2,3-$^{13}C_3$]pyruvate, were incorporated into polyketide moieties (Fig. 2E, J_{C7-C8} = 36 Hz, J_{C3-C4} = 46 Hz). Succinic acid is the third candidate since it was reported to be incorporated into an α-tetronic acid containing compound, caloric acid.[11] Feeding experiment of [1,4-$^{13}C_2$]succinic acid to the producing strain of **1** resulted in the absence of peak enhancement in not only this C_3 unit but also other substructures in **1**, supporting that C_4 unit is not utilized by actinomycetes. Consequently, the α-acyltetronic acid moiety of **1** is deduced to be composed of an acetate and a glycerol units. These results indicated that only glycerol, which might be converted to glyceric acid but not pyruvic acid was utilized as the intermediate of biosynthesis of the C_3 unit of the α-acyltetronic acid moiety. Feeding experiment of pyruvic acid and succinic acid suggested that downstream metabolites of glycerol do not revert to be precursors of the α-acyltetronic acid moiety. By summarizing these observations, the α-acyltetronic acid moiety might be biosynthesized through acaterin like intermediate.[9]

This is the first report of the biosynthetic studies on the α-acyltetronic acid substructure in macrocyclic compound from actinomycetes. Although few studies on the biosynthesis of tetronic acid moieties in small compounds were carried out,[8–12] the biosynthetic genes of tetronic acid have never been isolated. We have established that the aglycone of **1** consists of two substructures, the α-acyltetronic acid and polyketide chain moieties. It is of interest but not still unclear that which moiety acts as the starter unit of this class of compounds. This problem is expected to be proved by the establishment of biosynthetic genes of **1**. Since the biosynthetic genes of secondary metabolites are clustered in actinomycetes,[13] it is expected that the biosynthetic genes encoding the tetronic acid moiety in **1** are expected to be identified by tracing the polyketide biosynthetic genes. The genetic studies on the biosynthesis of **1** are now under way.

Acknowledgements

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References

1. Park, H.-R.; Furihata, K.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron Lett*. **2002**, *43*, 6941–6945.
2. Kaufman, R. J. *Genes Dev*. **1999**, *13*, 1211–1233.
3. Yoshida, H.; Haze, K.; Yanagi, H.; Yura, T.; Mori, K. *J. Biol. Chem*. **1998**, *273*, 33741–33749.
4. Roy, B.; Lee, A. S. *Nucleic Acids Res*. **1999**, *27*, 1437–1443.
5. Yun, J.; Tomida, A.; Nagata, K.; Tsuruo, T. *Oncol. Res*. **1995**, *7*, 583–590.
6. Jamora, C.; Dennert, G.; Lee, A. S. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7690–7694.
7. Lee, A. S. *Trends Biochem. Sci*. **2001**, *26*, 504–510.
8. Tamaoki, T.; Tomita, F. *J. Antibiot*. **1983**, *36*, 595–598.
9. Sekiyama, Y.; Araya, H.; Hasumi, K.; Endo, A.; Fujimoto, Y. *Tetrahedron Lett*. **1998**, *39*, 6233–6236.
10. Sekiyama, Y.; Fujimoto, Y.; Hasumi, K.; Endo, A. *J. Org. Chem*. **2001**, *66*, 5649–5654.
11. Reffstrup, T.; Boll, P. M. *Acta Chem. Scand., Ser. B* **1980**, *34*, 653–659.
12. Suga, T.; Hirata, T. *Bull. Chem. Soc. Jpn*. **1982**, *55*, 1582–1587.
13. Omura, S.; Ikeda, H.; Ishikawa, J.; Hanamoto, A.; Takahashi, C.; Shinose, M.; Takahashi, Y.; Horikawa, H.; Nakazawa, H.; Osonoe, T.; Kikuchi, H.; Shiba, T.; Sasaki, Y.; Hattori, M. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12215–12220.